

## **Development of Screening Methodologies for the Detection of Nanoscale Silver in FDA Regulated Food Products Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS)**

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### **Abstract**

This laboratory information bulletin (LIB) reports a method for the screening of total silver content in multiple fresh food commodities, such as blueberries, cranberries, fruit juice concentrate, carrots, broccoli, avocados, and shrimp. Each commodity was fortified with 35 nm silver, 100 nm silver, or silver nitrate solution. The samples were analyzed with a combination of microwave assisted acid hydrolysis and inductively coupled plasma – mass spectrometry (ICP-MS) to determine the silver concentration. The results indicate that the method produces recoveries of greater than 79% for all matrices. Furthermore, the particle size and/or stabilizer coating on the nanosilver does not appear to impact the validity of this method. The limit of quantitation (LOQ) for this method is 1.12 ng/g silver and the limit of detection (LOD) is 0.335 ng/g silver. Levels of environmental or background silver were not detected within any of the matrices analyzed during the method development.

Note: The Laboratory Information Bulletin is a tool for the rapid dissemination of laboratory methods (or information) that appear to work. It may not report completed scientific work. The user must assure him/her by appropriate validation procedures that LIB methods and techniques are reliable and accurate for his/her intended use. Reference to any commercial materials, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the Food and Drug Administration.

## Introduction

Nanotechnology is broadly defined as the study of matter at the atomic scale, in which a single dimension does not exceed 100 nanometers [1]. To date, the FDA has yet to establish or recognize a formal definition of nanotechnology. The scientific significance of this technology is based on the observation that the chemical and physical properties for a given element can be quite different depending on its size, especially as the size decreases into the nanoscale range. Furthermore, it has been documented that nano scale materials often exhibit unique or novel properties, when compared to their micro scale counterparts.

The inclusion of nano scale metals in various FDA regulated products has generated a great deal of interest from both the public and multiple FDA Centers [2,3]. While a complete toxicological profile for common earth metals has been fairly well documented, the advent of technologies to manipulate the size, geometry, and surface chemistry of these metals has generated concern related to the potential physiological impact on humans and animals [3]. It has been recently documented that over 1000 consumer products now contain nanomaterials, many of which are nanoscale metals.

Due its notoriety and relative ease of synthesis, this bulletin will focus on the development of a screening method for the determination of nanoscale silver in a variety of aqueous solutions and fresh perishable food products. The United States Environmental Protection Agency (EPA) recently proposed the conditional approval of nanosilver for use as an antimicrobial pesticide [EPA-HQ-OPP-2009-0936; FRL-8806-9], thus creating the potential for future regulation and/or tolerance levels within foodstuffs. Due to its antimicrobial properties, it has been theorized that nanoscale silver could increase shelf life of a product if utilized as a coating on fresh produce, a component of packing materials and films, or as an ingredient in brine coating used to quick freeze seafood.

The ionic forms of silver are known to exhibit cytotoxic effects to humans, with the degree of toxicity depending on the dosage, route of exposure, and chemical composition. Furthermore, silver can potentially impact human health by causing immune deficiency and skin discoloration, known as Argyria [4,5].

The goal of this study was to develop a method for the determination of total silver content, which would consist of the sum of any nanoscale silver and ionic silver salts, within several FDA regulated food commodities. This methodology is based on the utilization of microwave assisted acid hydrolysis and inductively coupled plasma - mass spectrometry. During the development of this method, we also intended to assess the inherent background levels of silver within the FDA regulated food commodities.

## Experimental

### Reagents:

- Reagent grade water (18 MΩ)
- Ultrapure Nitric Acid (JT Baker Ultrex II)
- Ultrapure Hydrochloric Acid (JT Baker Ultrex II)
- Silver 1000 µg/mL in 3% HNO<sub>3</sub> ICP-MS single element standard (Ricca, SPEX, SPE Sciences)
- Indium 10 µg/mL in 2% HNO<sub>3</sub> ICP-MS single element standard (Ricca, SPEX, SPE Sciences)
- Silver Nitrate (Acros - Ultrapure grade)

### Apparatus:

- Inductively Coupled Plasma - Mass Spectrometer (ICP-MS) – Agilent 7700x with Octopole reaction system, MassHunter software version B.03.001, Mira Mist Nebulizer.
- Microwave Accelerated Reaction System - CEM MARSXpress, 15mL Teflon vessels
- Vessel capping station - CEM MARSXpress capping station
- Analytical balance - Mettler AX504

### Supplies:

- 15mL polypropylene tubes (Sarstedt #62.554.205)

### Intermediate Standards Preparation:

1. Silver Intermediate Standard 1 (10,000 µg/L): Transfer 1.00 mL of the 1000 µg/mL silver stock standard to a clean 100 mL volumetric flask. Add 5.00 mL of ultrapure nitric acid and dilute to volume with reagent grade water.
2. Silver Intermediate Standard 2 (100 µg/L): Transfer 1.00 mL of the silver intermediate standard 1 to a clean 100 mL volumetric flask. Add 5.00 mL of ultrapure nitric acid and dilute to volume with reagent grade water.

### Working Standards:

Silver Concentration (µg/L)	Volume of Silver Intermediate Standard 1 (mL)	Volume of Silver Intermediate Standard 2 (mL)	Volume of 10 µg/mL Indium Stock Solution (mL)	Volume HNO <sub>3</sub> (mL)	Volume HCl (mL)
0	0	0	1.00	10.0	5.00
1.00	0	1.00	1.00	10.0	5.00
50.0	0.500	0	1.00	10.0	5.00
100	1.00	0	1.00	10.0	5.00
250	2.50	0	1.00	10.0	5.00
500	5.00	0	1.00	10.0	5.00

All working standards are diluted to a final volume of 100 mL in a class A volumetric flask.

**Sample Fortification:**

The single laboratory validation of this method utilized three unique spiking solutions cited below. Each solution was spiked on the matrices of interest at a level of 0.500 ppm, 12.5 ppm, and 25.0 ppm.

1. 35 nm silver - synthesized in-house using published methodology [6]. See Appendix 1 for characterization information.
2. 100 nm silver - synthesized in-house using published methodology [7]. See Appendix 1 for characterization information.
3. 1000 µg/mL Silver Nitrate solution - 157.5 mg of ultrapure silver nitrate dissolved into 100 mL of 2% nitric acid in a 100mL volumetric flask.

**Independent Calibration Verification (ICV):**

From a different source of 1000 µg/mL silver, prepare a solution with a final concentration of 100 µg/L silver and 100 µg/L indium. This solution should contain 10% HNO<sub>3</sub> and 5% HCL so that it exhibits an equivalent acid composition relative to the working standards.

**Continuous Calibration Verification (CCV):**

It is suggested that the 50 µg/L working standard be used as a CCV.

**Certified Reference Material (CRM):**

Non-defatted lobster hepatopancreas LUTS-1 (National Research Council Canada, <http://www.nrc-cnrc.gc.ca>). The CRM was prepared according to the manufacturers specifications, which included drying the materials to remove the moisture content.

**Test Sample Preparation:**

1. Accurately weigh  $0.20 \pm 0.04$  grams of sample directly into the microwave digestion vessel. Record the sample weight.
2. Matrix spikes only: pipette the appropriate volume of ionic or nanosilver test article and mix well. This mixture should be allowed to rest for at least one hour.
3. Dispense 3.0 mL nitric acid and 1.5 mL of hydrochloric acid into each vessel.
4. Seal each vessel with capping station.
5. Digest at 1200 W 100% power, ramp to 180° C in 15 minutes hold for 15 minutes, followed by 1200 W 100% power ramp to 200° C in 10 minutes and hold for 20 minutes (Table 1).
6. Allow sample to cool for one hour.
7. Quantitatively transfer the digested solution to a clean 15 mL polypropylene tube.
8. Wash the digestion vessel with deionized water and transfer the wash to the 15mL polypropylene tube.

9. Add 100 µl of 10 µg/mL Indium solution in 3% nitric acid.
10. Dilute each sample to a total volume of 10 mL.

### Instrumental Parameters

All samples were analyzed using an Agilent 7700 ICP-MS system. Prior to analysis, the system was tuned in accordance with the manufacturer's specifications. Data was acquired for both  $^{109}\text{Ag}$  (silver) and  $^{115}\text{In}$  (indium) using three points per peak and an integration time of 0.30 seconds per point and 0.90 seconds per mass. Between each sample, the system was rinsed with 2% nitric acid to prevent any carry over effects.

### Calculations

- A calibration curve was generated using linear regression,  $Y = mX + Z$ , where Y is ratio between count per second of analyte (Silver) and count per second of internal standard (Indium), X is analyte (Silver) concentration in µg/g, and Z is the Y-intercept. The total dilution factor is 50, thus should be included in the final calculation of silver concentration.
- Method Detection Limit,  $\text{MDL}^a = 3 \times \text{standard deviation of the method blank}$
- Limit of quantitation,  $\text{LOQ}^b = 10 \times \text{standard deviation of method blank}$
- Percent recovery =  
$$\frac{\text{experimental concentration } (\mu\text{g/g}) - \text{matrix background } (\mu\text{g/g})}{\text{theoretical concentration } (\mu\text{g/g})} \times 100$$

<sup>a</sup> calculated using CFR 40 CFR, Part 136, Appendix B

<sup>b</sup> calculated using 10X standard deviation of method blank (n=7)

### Suggested Analytical Batching Protocol:

An analytical batch should consist of the following quality control samples:

1. Two laboratory control blank (matrix blank).
2. Two method control blank (reagent blank).
3. Two fortified laboratory control blanks (matrix spikes)
4. Two certified reference materials (CRM)
5. An independent calibration verification standard (ICV).
6. One continuing calibration verification standard (CCV) for every six samples analyzed.

### Quality Control Limits:

- ICV and CCV solution should be within  $\pm 5\%$  of the prepared concentrations.
- Matrix spike recoveries should be within  $\pm 20\%$  of the prepared concentrations.

- Certified reference material should be within two standard deviations of the published values.
- The calibration curve should exhibit a correlation coefficient of 0.995 or greater.

## Results and Discussion

The key to the development of a successful method for the determination of silver can be directly attributed to the sample preparation, primarily the optimization of the acid dissolution procedure. The ratio of nitric and hydrochloric acids can impact both the dissolution efficiency of the sample, as well as the solubility of silver ions in solution. Based on the definition from the NNI [1] two nanoparticles (35 and 100 nm), as well as silver nitrate, were selected to demonstrate the methods capability with respect to dissolution.

Our initial experiments utilized only nitric acid, which provided excellent dissolution efficiency for the various food matrices; however, the total silver recovery was relatively low (>80%). During the methods development process, we also determined that the use of high percentages of hydrochloric acid provided excellent silver recovery, but poor matrix dissolution. We attribute the increased efficiency of the nitric acid (120.5 °C) to its' higher boiling point when compared to hydrochloric acid (84 °C).

The recovery issues of silver can be attributed to the fact that silver halides are generally very insoluble ( $\text{AgCl } K_{\text{sp}} = 1.77 \times 10^{-10} \text{ mol l}^{-1}$ ). Considering that many foods have some naturally occurring levels, it is theorized that the silver is immediately ionized by the acid, then subsequently reacts with the halides to form insoluble salts. The silver halides precipitates can be dissolved in the presence of excess hydrochloric acid, resulting in a water soluble silver dichloride complex anion ( $\text{Ag}(\text{Cl}_2)^-$ ) [8].



Since the formation and stability of water soluble complexes is dependent on chloride ion concentration, it is important to optimize the ratio of hydrochloric and nitric acids [6]. For this method, the ratio of the two acids was determined to be 2:1 (nitric: hydrochloric).

Table 1 indicates the appropriate parameters for sample dissolution using the CEM MARSPress microwave assisted dissolution system. A variety of fruits, vegetables, and shrimp were processed using the aforementioned acid composition and microwave parameters. At the completion of the microwave program, no solid material remained. All samples were clear, which is significant for commodities which contain high lipids content, such as avocados.

With respect to the inductively coupled plasma - mass spectrometry (ICP-MS) analysis, many parameters were evaluated to determine the optimum instrumental parameters. Modern ICP-MS instruments have the unique ability to use collision cell technologies to decrease interferences associated with matrices and polyatomic species. For this study, we collected data using three unique modes within the ICP-MS system: no collision cell gas, utilization of helium as a collision gas, and utilization of hydrogen as a collision gas. It was determined that the utilization of a collision cell gas for the analysis of silver was not advantageous. Thus we have only reported the results obtained using no collision cell gas, primarily because those results provided the lowest instrumental limits of detection and quantitation.

To ensure that fluctuations associated with sample introduction into the ICP-MS system did not impact the validity of this method, we utilized Indium as an internal standard. Indium was selected due to its similarity in atomic mass when compared to silver. The internal standard was included in the calibration standards and was added to each analytical and quality control sample.

The combination of the optimized sample preparation and instrumental method resulted in a robust and reproducible method for the determination of total silver content within a variety of fresh food commodities. Table 2 provides recovery data on fruits, vegetables, and shrimp which have been fortified with starch stabilized 35 nm silver. Average percent recoveries ranged from 80 – 95%, with no appreciable difference between types of sample or fortification level. Seven replicates of each sample were analyzed at each level of fortification to generate meaningful statistics related to the methods variability. Table 3 provides recovery data on the same matrices which have been fortified with polyvinylpyrrolidone (PVP) stabilized 100 nm silver. Average percent recoveries ranged from 81 – 103%, with no appreciable difference between types of sample or fortification level. Seven replicates of each sample were analyzed at each level of fortification to generate meaningful statistics related to the methods variability. Characterization data on each of the nano silver fortification samples can be found in Appendix A.

The use of an ionic silver control was vital to demonstrate that no appreciable difference would occur between ionic and non-ionic (nano) silver. Table 4 provides recovery data of the fortification of silver nitrate solution on the same fruits, vegetables, and shrimp which were used in the nanosilver fortification experiments. The average percent recoveries ranged from 79 – 92%, which is very similar to the recoveries obtained when analyzing the products fortified with nanosilver. Seven replicates of each sample were analyzed at each level of fortification to generate meaningful statistics related to the methods variability.

It is important to note that multiple unfortified matrix blanks were analyzed using this methodology. The results of these analyses are presented in Table 5. For all matrices and analyses, no background level was detected at or above the



method detection limit (MDL) of 0.335 ng/g. This is significant in that one of the current knowledge gaps is the unknown contribution of environmental factors toward the total silver concentration of fresh fruits and vegetables. Based on the results presented in this LIB, it appears any level of silver which is identified within such fruits and vegetables could be considered suspicious and warrant further analytical testing to determine its origin.

A summary of the statistical limits of the method are located in Table 6. They indicate that limit of quantitation (LOQ) for this method is 1.12 ng/g silver and the limit of detection (LOD) is 0.335 ng/g silver. Currently the only regulatory limit for silver intake is 5 µg/kg body weight in drinking water, as dictated by the United States Environmental Protection Agency (US EPA). Based on this limit, it seems reasonable that this methodology will be capable of addressing future FDA regulatory requirements.

To complete the validation of the method we analyzed non-defatted lobster hepatopancreas (LUTS1), which is a commercially available certified reference material (CRM) from the National Research Council Canada. Unlike our process of sample fortification, the silver content of this product is naturally occurring. It has been well documented that marine organisms tend to accumulate heavy metals, such as silver, within their natural environment [9]. The results of our analysis presented in Table 7, indicate that the method is both accurate and precise for the determination of environmentally incurred silver. We based our quality control parameters at two times the published standard deviation ( $\pm 2SD$ ), which is generally regarded as an acceptable limit. Over the course of the method validation, we performed a total of 50 analyses on the LUTS1 CRM. The results of these analyses never exceed the acceptability criteria of  $\pm 2SD$ , which indicates that the methodology is valid for the determination of total silver content.

## Conclusion

The quantitative analysis of nanosilver offers unique challenges due to the inclusion of stabilizing coatings and limited solubility in solution upon ionization. When included in a complex matrix, such as food, the challenges of solubility increase due to naturally occurring anions, such as chloride and sulfate. The results presented in this publication indicate that such challenges can be overcome when optimizing both the sample dissolution and the ICP-MS parameters.

This method has been validated using both ionic silver and two sizes of nanosilver. Since such a method does not currently exist within the FDA Elemental Analysis Manual (EAM), methodology such as this would be advantageous in the event that a regulatory issue involving silver or/and nanosilver comes to fruition.



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**Table 1: Microwave Parameters**

	Max Power	% Power	Ramp (min)	Temperature °C	Hold (min)
1	1200W	100	15	180	15
2	1200W	100	10	200	20

**Table 2: Spike Recoveries 35 nm Silver (n=7)**

	0.50 µg/g	12.5 µg/g	25 µg/g
Blueberry	93.1% (±4.36)	90.8% (±0.67)	92.7% (±0.69)
Cranberry	90.7% (±1.60)	91.6% (±0.61)	90.8% (±0.93)
Fruit juice concentrate	94.3% (±0.62)	94.6% (±0.27)	94.3% (±0.53)
Carrot	89.3% (±0.74)	89.3% (±1.02)	90.1% (±0.60)
Broccoli	80.9% (±0.99)	83.4% (±0.78)	82.5% (±1.10)
Avocado	81.3% (±0.65)	84.5% (±1.10)	85.6% (±0.81)
Shrimp	84.9% (±0.41)	86.2% (±1.68)	86.0% (±0.71)

**Table 3: Spike Recoveries 100 nm Silver (n=7)**

	0.50 µg/g	12.5 µg/g	25 µg/g
Blueberry	96.6% (±1.94)	103% (±1.32)	101% (±1.72)
Cranberry	92.3% (±0.58)	99.1% (±0.27)	99.8% (±0.33)
Fruit juice concentrate	90.1% (±2.72)	92.8% (±0.67)	93.7% (±0.54)
Carrot	93.1% (±0.75)	98.4% (±0.68)	98.5% (±0.45)
Broccoli	81.4% (±0.74)	86.1% (±0.82)	85.4% (±0.96)
Avocado	81.0% (±1.00)	87.9% (±0.69)	88.8% (±1.40)
Shrimp	89.3% (±1.01)	95.9% (±0.63)	96.7% (±0.39)

**Table 4: Spike Recoveries Ionic Silver (n=7)**

	0.50 µg/g	12.5 µg/g	25 µg/g
Blueberry	87.9% (±0.92)	90.3% (±0.56)	90.3% (±0.32)
Cranberry	90.7% (±1.60)	91.6% (±0.61)	90.8% (±0.93)
Fruit juice concentrate	84.6% (±0.32)	86.4% (±1.23)	86.6% (±0.52)
Carrot	84.0% (±0.49)	85.4% (±0.23)	85.7% (±0.42)
Broccoli	79.2% (±0.82)	80.4% (±1.40)	81.0% (±1.20)
Avocado	79.7% (±0.52)	82.5% (±0.54)	82.9% (±0.54)
Shrimp	82.9% (±0.42)	84.3% (±0.86)	84.4% (±0.40)

**Table 5: Background Environmental Silver**

Matrix	Concentration
Blueberry	None detected
Cranberry	None detected
Fruit juice concentrate	None detected
Carrot	None detected
Broccoli	None detected
Avocado	None detected
Shrimp	None detected

**Table 6: Limits of Detection and Quantitation**

Analyte	MDL <sup>a</sup> (ng/g)	LOQ <sup>b</sup> (ng/g)
Silver (Ag)	0.335	1.12

<sup>a</sup> calculated using CFR 40 CFR, Part 136, Appendix B

<sup>b</sup> calculated using 10X standard deviation of method blank (n=7)

**Table 7: Certified Reference Material, LUTS-1 (n=50)**

Analyte	Mean (µg/g)	Certified Value (µg/g)	Acceptance Criteria (µg/g)
Silver (Ag)	3.97 (±0.38)	3.89 (±0.33)	3.23 – 4.55

*The acceptance criteria were based on two times the published standard deviation (±2SD).*

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## Appendix A

Nanoparticle characterization was performed in accordance with the published methods. For both the 35 nm and 100 nm silver particles, this consisted of a determination of particle size, evaluation of the spectroscopic properties, and assessment of the concentration of silver.

### Particle Size

The size of each nanosilver fortification sample was determined via transmission electron microscopy (TEM). A 5  $\mu\text{L}$  sample of each nanosilver test article was placed on a silicon dioxide coated formvar/Cu 200 mesh grid and vacuum dried within a desiccator for 24 hours. Images were collected with JEOL GEM 2100 LaB<sub>6</sub> TEM operated at a 200 kV acceleration voltage. The TEM instrument was equipped with a high resolution four megapixel digital camera. Images were processed with NIH image-J software for size statistics, where we randomly selected 100 nanoparticles in order to determine the average particle diameter.

Electron microscopy is the ideal technique for the measurement of the metallic core of the nanoparticle, since its imaging technology is based on electron density. Since silver exhibits a much higher electron density compared to the stabilizing agents (starch and PVP), an accurate core shell particle size can be reported.

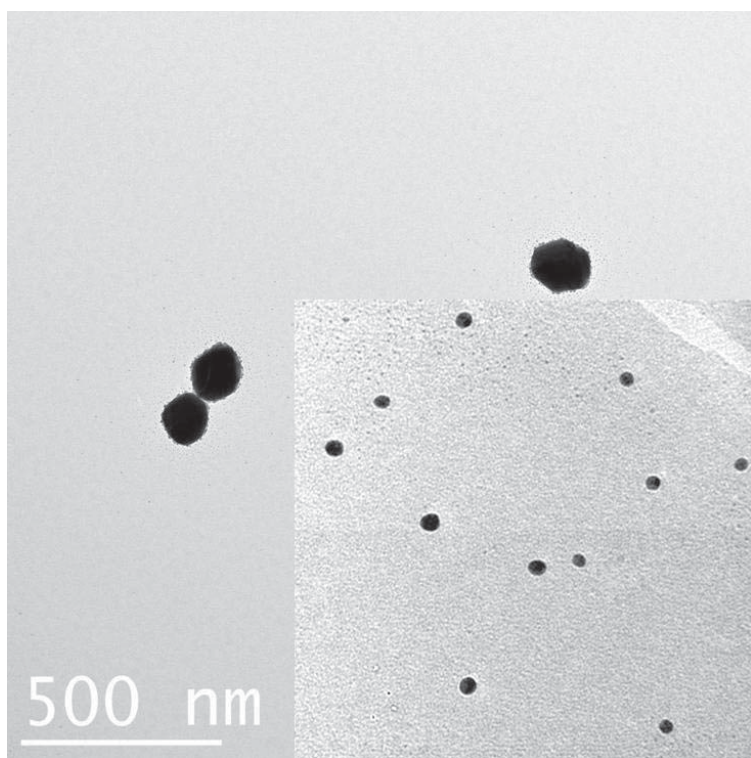


Figure 1: TEM image of the 35 nm (inset) and 100 nm silver particles.

### Spectroscopic properties

UV/Vis absorbance spectra of nanosilver samples were collected with a Perkin Elmer Lambda 35 spectrometer using 1 cm path length quartz cuvette. The peak absorbance for the 35 nm silver was 412 nm, while the peak absorbance for the 100 nm silver was 434 nm. These peak absorbance values are consistent with published values, and display the red shift typically associated with an increase in the particle size of nanosilver.

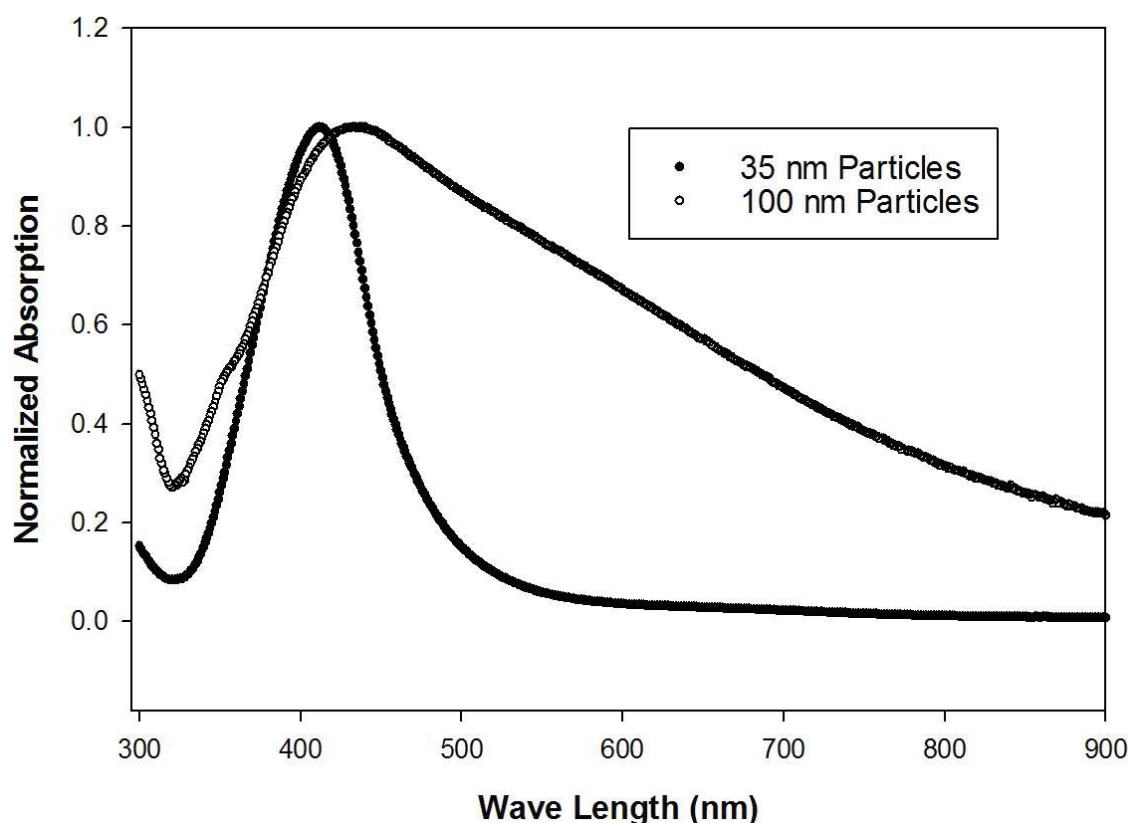


Figure 2: Absorbance spectra of the 35 nm and 100 nm silver particles.

### Concentration

Each nanosilver fortification sample was assayed using an Agilent 7700x ICP-MS to determine the total silver concentration within each test article. The 35 nm silver was determined to be 48.8  $\mu\text{g/g}$ ; while the 100 nm silver was determined to be 56.5  $\mu\text{g/g}$ .